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Research article

Cultivating a greener future: Exploiting trichoderma derived secondary metabolites for fusarium wilt management in peas

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ABSTRACT

This study aimed to identify efficient *Trichoderma* isolate(s) for the management of Fusarium wilt in peas. Four different pea germplasms (Sarsabz, Pea-09, Meteor and Supreme) were evaluated for resistance against *Fusarium oxysporum* in pot assay. Resistant germplasm exhibits a varying range of disease severity (23%) and percent disease index (21%), whereas susceptible and highly susceptible germplasm exhibit maximum disease severity (44–79%) and percent disease index (47–82%). The susceptible germplasm Meteor was selected for *in vivo* experiment. Five different *Trichoderma* spp. (*Trichoderma koningii*, *T. hamatum*, *T. longibrachiatum*, *T. viride*, and *T. harzianum*) were screened for the production of hydrolytic extracellular enzymes under *in vitro*. In-vitro biocontrol potential of *Trichoderma* spp. was assayed by percentage inhibition of dry mass of *Fusarium oxysporum pisi* (FOP) with *Trichoderma* spp. metabolite filtrate concentrations. Maximum growth inhibition was observed by *T. harzianum* (50–89%). *T. harzianum* metabolites in filtrate conc. (40%, 50%, and 60%) exhibited maximum reduction in biomass and were thus used for *in vivo* management of the disease. The pot experiment for in-vivo management also confirmed the maximum inhibition of FOP by *T. harzianum* metabolites filtrate at 60% by reducing disease parameters and enhancing growth, yield, and physiochemical and stress markers. *Trichoderma* strains led to an increase in chlorophyll and carotenoids (34-26%), Total phenolic 55%, Total protein content 60%, Total Flavonoid content 36%, and the increasing order of enzyme activities were as follows: CAT > POX > PPO > PAL in all treatments. These strains demonstrate excellent bio-control of Fusarium wilt in pea via induction of defense-related enzymes. The present work will help use *Trichoderma* species in disease management programme as an effective biocontrol agent against plant pathogens.

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1. Introduction

Plant diseases threaten food security by reducing crop yield. One of the most harmful diseases to peas (*Pisum sativum* L.) worldwide, Fusarium *wilt* is brought on by *Fusarium oxysporum* f. sp. *pisi* (Fop), which results in significant yield losses in all pea-growing fields [1] A common soil-borne fungus called *Fusarium oxysporum* (FOP) has morphologically similar plant-pathogenic and non-pathogenic strains [2]. *Fusarium* species-caused wilt diseases have a significant impact on agricultural production globally as well as in Pakistan [3]. The most prevalent and harmful specie of the *Fusarium* genus is *Fusarium oxysporum* which infects a variety of host plants [4]. The pathogen *Fusarium oxysporum* Schl. f.sp. *pisi* (Snyd & Hans) is regarded as a danger to Pakistan's and the world's pea crop in general [5]. Depending on the current climatic circumstances, this disease might result in a partial or total loss of the pea crop [6,7]. *Fusarium* wilt is among the most serious diseases affecting grain legumes. Field pea (*Pisum sativum*) is affected by *Fusarium oxysporum* f. sp. *pisi* (FOP), a significant and harmful disease [8]. It is a pathogen that resists treatment and can persist in soil for up to 30 years [9]. For instance, the diseases *Fusarium oxysporum* f.sp. *pisi*, *ciceris*, *lentils*, *phaseoli*, and *medicaginis* successively cause destruction in pea, chickpea, lentil, common bean, and alfalfa respectively throughout the world [10]. *Fusarium* wilt root rot substantially reduce pea crop output and may result in 10–50% crop loss each year in Pakistan [11].

Antagonistic organisms, including several species of Trichoderma, are strategically employed to regulate soil-borne diseases, fostering a symbiotic relationship critical for ecological balance and disease control. According to the benefit-cost ratio [12], the use of antagonistic species is an environmentally conscious, ecologically sound, and economically viable technique for reducing environmental danger and toxicity. The biological management of diseases is becoming more important than other approaches in the current world. Antagonists from the rhizosphere of the host plants were identified for the treatment of soil- and seed-borne diseases. Biocontrol chemicals offer not only effective disease control, but also safe and environmentally sustainable alternatives. The concept of biocontrol refers to the introduction of antagonists into cropping systems. A living proliferating biocontrol agent can provide ongoing disease control without the use of chemicals. Furthermore, chemical methods may cause an imbalance in the microbial population, reducing the activity of beneficial organisms [13]. As a result, the direct application of antagonists would be a safer way to introduce microorganisms into the soil for the biological control of soil-borne plant diseases. It has long been recognized that biocontrol agents produce a variety of antibiotics and parasitize other harmful fungi [14]. Among biocontrol agents, *T. viride* has been extensively reported effective in fighting soil-borne infections in field crops, notably against *Fusarium* wilt [15]. *Trichoderma* is a genus of asexually reproducing filamentous fungus that is widespread across the world [16]. *Trichoderma* spp. are filamentous fungi that live in soil and include species that are hostile to plant infections such as *Pythium* spp., *Fusarium* spp., and others. Pathogen growth inhibition is achieved by a variety of processes, including the development of lytic enzymes and antibiotics, as well as significantly faster space growth and food consumption [17]. Different isolates of *Trichoderma* spp., such as *T. asperellum*, *T. harzianum*, and *T. viridie*, have shown their greatest effectiveness against various diseases, especially soil-borne infections [18].

They can also be found on the root surfaces of numerous plants and on rotting bark, particularly when other fungi have harmed the bark [19]. Trichoderma species have long been recognized as biological control agents (BCAs) for preventing and treating plant diseases, and for promoting the growth and development of plants by releasing a wide variety of secondary metabolites i.e., both volatile and nonvolatile (diffusible), negatively impact various fungi [20]. *Trichoderma*-based formulations are the most effective bio-fungicides in integrated pest control, accounting for more than 60% of all registered bio-fungicides [21]. They are now employed as commercial biological control agents for fungal and fungal-like root rot pathogens such *R. solani*, *Pythium* spp., and *Fusarium* spp [22]. Several research has looked into the antagonistic activity of *Trichoderma* spp. against *F. solani* and *R. solani* in order to determine their efficacy as biocontrol agents and understand their antagonistic processes [23].

The objectives of research were 1) to assess the response of different pea germplasms against fusarium wilt disease through disease parameter viz., growth indexes, physio-chemical and enzymatic attributes; 2) *In-vitro* evaluation of antagonistic effect of different potential *Trichoderma* strains metabolites concentrations against *Fusarium oxysporum* f.sp. *pisi*;0.3) disease management potential of most potent *Trichoderma* strain metabolites concentrations through disease suppression, growth indexes, physiochemical and enzymatic attributes.

2. Materials and methods

2.1. 1Pathogen isolation and evaluation of pea (Pisum sativum) germplasm against Fusarium oxysporum f. sp. pisi

The pathogen *Fusarium oxysporum* f. sp. *pisi* (FOP) (FCBP-PTF-866) pure culture was procured from the first fungal culture bank of Pakistan (FCBP), Faculty of Agriculture Sciences, University of the Punjab, Lahore. The pathogen was then sub cultured aseptically on potato dextrose agar (PDA) medium in petri dishes. The fungal stock culture was prepared in 30% glycerol and then stored in the refrigerator. Four peas genotype (Sarsabz, Pea-09, Meteor and Supreme) were obtained from Ayyub Agriculture Research Institute (AARI), Faisalabad for its evaluation against pathogen. Seeds were soaked and after one day surface sterilized with 2% sodium hypochlorite solution and kept in petri plates until germination [24]. For the *in vivo* pathogenicity test, disposable cups were filled with sterilized soil. Pots (7.62 cm) were inoculated with freshly prepared inoculum of *Fusarium oxysporum* f. sp. *pisi* (10 mL pot^{-1} with a spore count (4.65×10^6 spores/mL). After 5 days, seeds were sown in inoculated soil, and pathogen-free soil was used as a control. The experiment was performed in a completely randomized design with three replicates of each inoculated and uninoculated germplasm [25]. After 40 days of inoculation wilting disease symptoms on pea plants were measured individually using 1–4 disease rating scale according to Ref. [26].

2.2. Isolation of antagonistic fungal isolates and management of Fusarium oxysporum under in vitro conditions through fungal metabolites

2.2.1. Extraction of Trichoderma metabolites

Five *Trichoderma* isolates (*Trichoderma koningii* (FCBP-PTF-765), *T. hamatum* (FCBP-SF-907), *T. longibrachiatum* (FCBP-SF-0083), *T. viride* (FCBP-SF-639), and *T. harzianum* (FCBP-SF-1277) were procured as potential biocontrol agents from the fungal biotechnology lab, Faculty of Agriculture Sciences, Lahore. The isolates were sub-cultured aseptically on potato dextrose agar (PDA) medium and incubated at 36 °C for 5–7 days. *Trichoderma* strains were sub-cultured on malt extract broth medium for metabolite production. *Trichoderma* strains plugs of about 5 mm were obtained from freshly growing margins of PDA cultures. The broth media was inoculated with each of the five *Trichoderma* strain plugs separately and incubated at 25 ± 2 °C for 20 days and the culture was filtered through sterilized Whatman filter paper in sterilized condition and the filtered culture broth was extracted as metabolites.

2.3. Bioassays from metabolites

Seven concentrations of each Trichoderma strain's metabolites, viz., 0, 10, 20, 30, 40, 50, and 60%, were prepared to test the antifungal activity of metabolites. 1.2% malt extract broth medium was prepared using the distilled water in each flask, viz., 60, 57, 51, 45, 39, 33, and 27 mL. Each concentration was prepared by adding 0, 3, 9, 15, 21, 27, 33 mL of metabolite filtrate to each flask containing to raise the final volume to 60 mL. Prepared concentrations of 60 mL were equally divided into three replicates containing 20 mL each in a 100 mL conical flask. All small flasks were simmered at 60 °C for 10–12 min without pressure to prevent contamination and then inoculated by using the plug method, i.e., one plug in each flask, and incubated at 28 °C for 7 days until growth in the control treatment reached its maximum.

2.4. Biomass determination and analysis

For biomass determination, pre-weighed filter papers were utilized. Broth cultures were filtered, and mycelial mat was collected. Biomass fresh weight was measured on a balance. The effectiveness of various concentrations of all metabolites was evaluated, and the increase or decrease in fungal biomass over the control was examined. Due to the usage of different concentrations, percentage biomass inhibition was calculated by using the formula [27].

2.5. Management of Fusarium oxysporum under in vivo conditions and disease assessment

The efficiency of potential *Trichoderma* strain (*T. harzianum*) concentrations and the fungicide Topsin M − 70 were evaluated for FOP-susceptible pea germplasm (MATEOR) in a pot trial. Pots were filled with soil and inoculated with 10 mL of *F. oxysporum* f. sp. pisi conidial suspension (4.65×10^6 spores/mL). After 10 days, AR10 metabolite concentrations of 40, 50, and 60% were used to inoculate the soil. The experiment was performed in a randomized complete block design. Pea seedlings (5 seeds per plant) were inoculated with pathogens as the positive control, while plants without pathogens served as the negative control. Percentage disease severity (DS) and Percentage severity index was calculated in term of percentage disease index according to the following formula [28].

2.6. Growth attributes and physiological parameters estimation

The plants were harvested after 40 and 80 days of pathogen inoculation for growth attribute assessment. The growth attributes such as plant height (PH), shoot length (SL), root length (RL), fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), dry root weight (DRW), number of pods (NOP), and number of seeds per pod (SPP) were measured to calculate the growth inhibition index (GII) (Khan et al., 2016). The growth inhibition index (GII), shoot length vigour index (SLVI), and shoot weight vigour index (SWVI) were measured. In pea plants, physiological attributes were assessed at the 40th and 80th days after pathogen inoculation. Total chlorophyll content, total carotenoid content, total phenolics, total protein content, and total flavonoid content of healthy and diseased leaves were assessed after 40 days.

2.7. Total chlorophyll, carotenoid, total phenolic, protein and flavonoid content

Pea leaves (0.5 g) were grounded (3 replicates per plant) in 80 percent ethanol. Leaf extract was centrifuged, and the supernatant was collected, which was used to calculate the photosynthetic pigments [total chlorophyll content (chlorophyll *a* + b) and carotenoids] (Shoaib et al., 2019). The absorbance of chlorophyll *a*, chlorophyll *b*, and carotenoids was measured at 645 nm, 663 nm, and 270 nm, respectively, using a spectrophotometer [29].

Total phenolics were determined in a reaction mixture consisting of 0.5 mL of ethanolic plant extract (3 samples per plant), 2.5 mL of 20% Na_2CO_3, and 0.025 mL of Folin-Ciocalteatou's reagent. After 45 min of incubation at 45 °C, the absorbance of samples was measured at 765 nm against a blank (without ethanolic plant extract) through a spectrophotometer [30].

0.5 g of pea leaves (3 samples per plant) were crushed in 5 mL of 0.1 M sodium phosphate buffer and centrifuged for 5 min at 10,000 rpm (4 °C). The supernatant was collected (0.1 mL) and mixed with the suitable reagent. Using the standard curve of bovine serum albumin, the total protein content was calculated at 650 nm [31,32].

1 mL of leaf extract was combined with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. 50 mg of quercetin was dissolved in 50 mL of methanol to make a stock solution. After 5 min, 0.3 mL of AlCl3 (10%) and 0.2 mL of NaOH (1 mol L^{-1}) were added to the

abovementioned solution, and the final volume was increased to 10 mL by adding additional distilled water. The absorbance was measured at 510 nm against a blank using a spectrophotometer to determine the flavonoids [32,33].

2.7. Enzymatic activities (catalase, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase activities)

The enzymatic activities of catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and urease were assessed in the pea leaves at the 40th and 80th days of pathogen inoculation.

The catalase activity was measured in a reaction mixture containing 0.1 mL of leaf enzyme extract and 2.9 mL of buffer B [0.05 M sodium phosphate buffer (pH 7.0) + 0.036 percent hydrogen peroxide (35 percent)]. A change in absorbance was measured at 240 nm at 30 s intervals for 3 min against a blank (3 mL of buffer B) [34].

For the peroxidase (POX) analysis, a reaction mixture consisting of 0.1 mL of leaf enzyme extract, 2.4 mL of 0.1 M phosphate buffer (pH 6 at 20 °C), 0.3 mL of 5.33% pyrogallol, and 0.2 mL of 0.5% hydrogen peroxide was used. For 5 min, the reaction mixture was incubated at 20 °C. The quantity of purpurogallin formed was calculated by comparing the absorbance at 420 nm to a blank [35].

Polyphenol oxidase activity was determined in a reaction mixture comprised of 0.1 mL of leaf enzyme extract, 2.3 mL of 0.05 M phosphate buffer (pH 6), and 0.6 mL of 10 mM pyrocatechol. The absorbance change was measured at 495 nm at 30 s intervals for 3 min against a blank [36].

PAL activity was estimated by measuring the amount of *trans*-cinnamic acid produced by the reaction of 0.4 mL enzyme extract with 1.1 mL of 0.1 mol L^{-1} sodium borate buffer (pH 8.8) and 0.5 mL of 0.012 mmol L^{-1} L-phenylalanine at 290 nm against blank [37].

3. Results

3.1. In-vivo experiment for screening of pea germplasm against Fusarium oxysporum f. sp. pisi

3.1.1. Disease assessment

Four pea germplasms were screened out against *Fusarium oxysporum* f. sp. *pisi*. The disease severity (DS) and percent severity index (PSI) exhibited statistically significant results for each inoculated genotype concerning its un-inoculated genotype. The results were evaluated after 40 and 80 days of pathogen inoculation. Germplasm Sarsabz was found in a highly resistant group and showed zero percent DS and PSI, respectively, compared to its healthy plants. The DS 18–21% and PSI 17–23% in germplasm pea-09 were observed

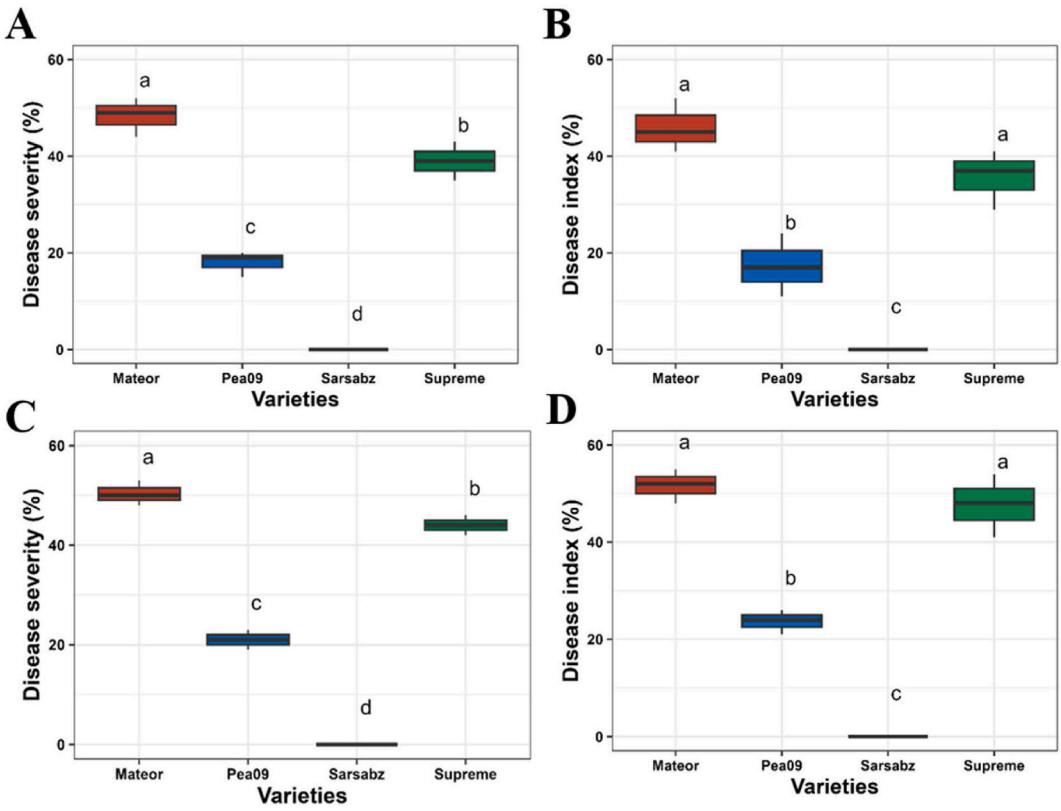

Fig. 1. (A–D): (A–B) showing disease severity (%) and percent disease index (%) after 40 days and (C–D) showing after 80 days for the assessment of Fusarium wilt in different peas germplasms.

significantly higher than highly resistant germplasm. While germplasm supreme exhibited significantly greater DS (39–44%) and PSI in the range of (35–47%). Highly susceptible germplasm (meteor) exhibited the highest disease severity and percent disease index in the range of 48 to 46% and 50–51% after 40 and 80 days of inoculation [Figs. 1(A–D) and 11].

3.2. In-vitro experiment for disease management

3.2.1. Dry biomass inhibition determination Trichoderma strains metabolites

Trichoderma strains metabolites were evaluated for their antifungal activity against *Fop*. Different concentrations (0 (control), 10, 20, 30, 40, 50, and 60%) of *Trichoderma* isolate metabolites were observed for dry biomass inhibition of the fungal pathogen. All concentrations of metabolites exhibited significantly variable data on fungal biomass inhibition. *T. harzianum* metabolite concentrations (40, 50, and 60%) were observed to reduce maximum fungal biomass. The minimum reduction in fungal biomass was significantly observed in *T. hamatum*, respectively, over control (Figs. 2 and 12A–D).

3.3. In-vivo experiment for disease management

The results of in-vitro experiment showed that highest concentrations of *T. harzianum* metabolites exhibited potential to reduce fusarium wilt disease and enhance the growth of susceptible germplasm (meteor). Six treatments were used under in-vivo experiment.

3.3.1. The effect of treatments on disease parameters: disease severity and disease severity index

Negative control did not exhibit any result of disease. The highest incidence of disease was observed in positive control with disease severity (74.26%) and (82.23%) and percent disease index (80.67%) respectively at 40 and 80 days of inoculation. Chemical fungicide (Topsin-M) treatment significantly reduced disease severity 43% and 47% and percent disease index 53%, 54%. From *T. harzianum* metabolites, treatment with 60% conc. exhibited significantly less disease severity (47%) and (55%) and percent disease index (56%), (57%). Treatment with metabolites conc. (40%) exhibited disease severity (66–74%) and percent disease index (69–81%). While, treatment with (50%) metabolites concentration exhibited disease severity (55–70%) and disease index (64–72%) respectively. The effect of chemical fungicide and *T. harzianum* metabolites conc. (60%) exhibited more or less similar results and found effective for the management of fusarium wilt disease [Figs. 3(A–D) and 10(A–E)].

3.4. Effect of treatments on plant growth

Root length was significantly affected in the positive control as compared to the negative control. The other *T. harzianum* metabolite concentrations (40% and 50%) caused a 45–60% increase in root length over the positive control. Root fresh and dry weights were increased (2.5 g and 0.5 g) when treated with *T. harzianum* metabolites at a concentration of 60%. With fungicide treatment, the studied attributes were considerably increased (2 g and 0.5 g). The shoot length of the plant with fungicidal treatment was observed, and improvement (50–66%) was calculated in the shoot length, dry biomass, and fresh biomass. The maximum improvement (70–85%) in shoot attributes was observed in soil application of *T. harzianum* metabolites (conc.) (40% and 50%). *T. harzianum* metabolites combined (40% and 60%) treatments exhibited considerably less improvement (40–60%) on shoot growth parameters, respectively.

Plant height was highly affected in positive control and reduced significantly (18 cm and 29 cm, respectively), and fungicide

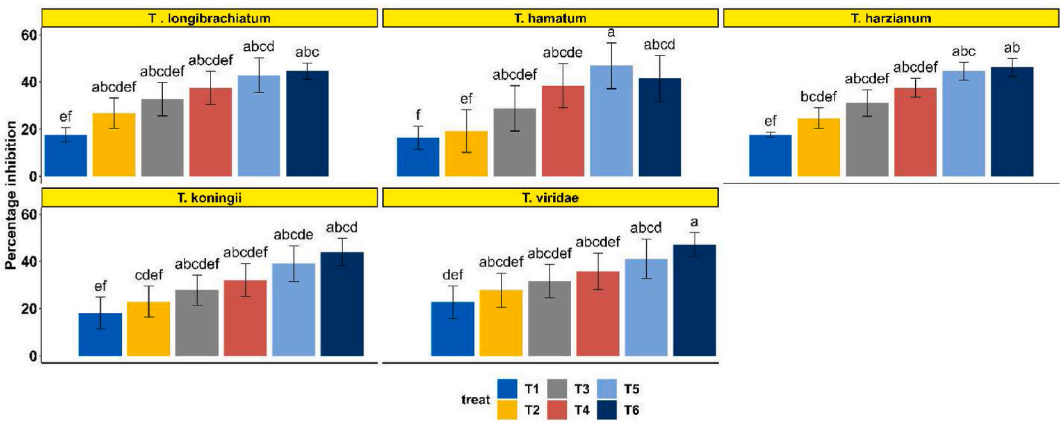

Fig. 2. In-vitro antagonist bioassay. Effect of different *Trichoderma* spp. metabolites on the percentage decrease in growth of *F. oxysporum* f.sp. *pisi* at different concentrations different *Trichoderma* spp. are, i.e., *T. longibrachiatum*, *T. hamatum*, *T. harzianum*, *T. koningii*, and *T. viridie*. Panels with different colors show different treatments from T1 to T6 where T6 represents control and means followed by the similar letters on each panel are not statistically different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

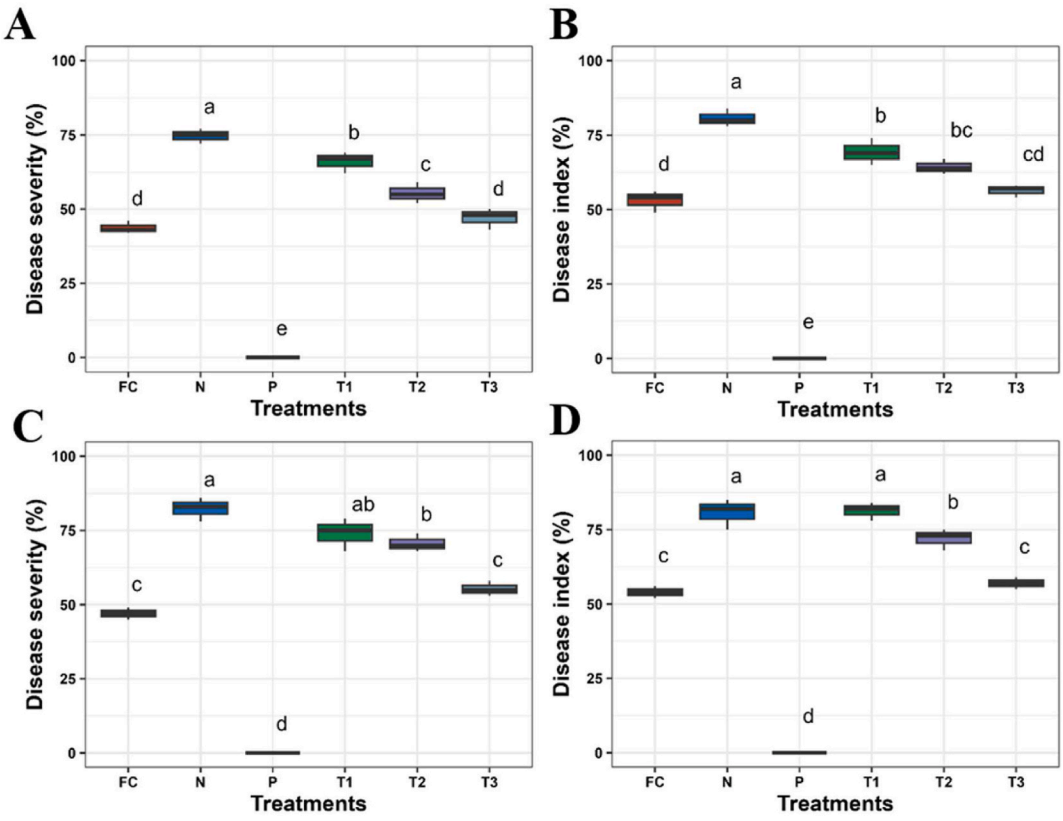

Fig. 3. (A–D): (A–B): Effect of *T. harzianum metabolites* con. (40%, 50%, and 60%) on disease severity % and percent disease index % (B) in susceptible germplasm (Meteor) against *F. oxysporum* f.sp. *pisi* 40 days after inoculation. (C–D) after 80 days of inoculation.

improved plant height (21 cm–35 cm). *T. harzianum* metabolites conc. (40% and 50%) and *T. harzianum* metabolites conc. (60%) exhibited significant improvement (23 cm–37 cm) in plant height at 40- and 80-day intervals. The number of pods in plant 1 with *T. harzianum* metabolites (60%) exhibited an increase of 75–90%. The maximum (67–75%) yield of number of pods per plant over *T. harzianum* metabolites (40–50%) was observed in soil application of chemical fungicide. *T. harzianum* metabolites conc. (60%, 405 and 60%) increased the number of seeds pod-1 by (75–90%), (40% and 50%), and (55–65%), respectively. Chemical fungicide treatment produced a 67–75% yield.

Growth inhibition index (GII) was considerably higher in negative control (10–14%) compared to positive control (7.4–9). The increase (10–13%) in growth inhibition index (GII) with conc. 60% *T. harzianum* metabolites (conc. 40% and 50%) exhibited an 8–11% and an 8–12% growth inhibition index (GII). The shoot length vigour index (SLVI) was significantly greater in the negative control (1614–2772) than in the positive control (1311–2349). Shoot length vigour index (SLVI) increased significantly (1967–3327) in *T. harzianum* metabolites (60%) at 40- and 80-day intervals, respectively. *T. harzianum* metabolites in the 40% and 50% treatment revealed (1517–2643) and (1729–2929), respectively. Chemical fungicides also showed a significant increase (1657–2708) in shoot length vigour index (SLVI). The shoot weight vigour index (SWVI) was substantially higher in the negative control (513–809) than in the positive control (332–461). *T. harzianum* metabolites (60%) greatly improved shoot weight vigour index (SWVI) (635–692), respectively. *T. harzianum* metabolites in 40 and 50% of treatments exhibited (462–539) and (515–597), respectively. Chemical fungicides significantly increased shoot weight and vigor index (551–556) (Figs. 4, 5 and 13).

The results of correlation analysis revealed that after 40 and 80 days of inoculation, there was a significant difference between agronomic parameters with different treatments. Negative and positive treatments displayed a strong negative correlation, and T2 (40%) and T3 (60%) are positively correlated (Fig. 6).

3.5. Effect on physio and biochemical attributes

Physiological and biochemical attributes were recorded after 40 and 80 days of inoculation, respectively. Total chlorophyll content was significantly reduced (34–26%) in T5 (*T. harzianum* metabolites conc. 60%) at 40 and 80 days of inoculation. A significant decline (52–42%) was observed in carotenoids as compared to the positive control. The phenolic contents were observed (54–55%) in T5. Phenolic content significantly decreased in inoculated plants as compared to their respective controls. Total protein content (TPC) was significantly increased in T5 (60%) and decreased in the positive control. The inoculated plant exhibited a significant decrease

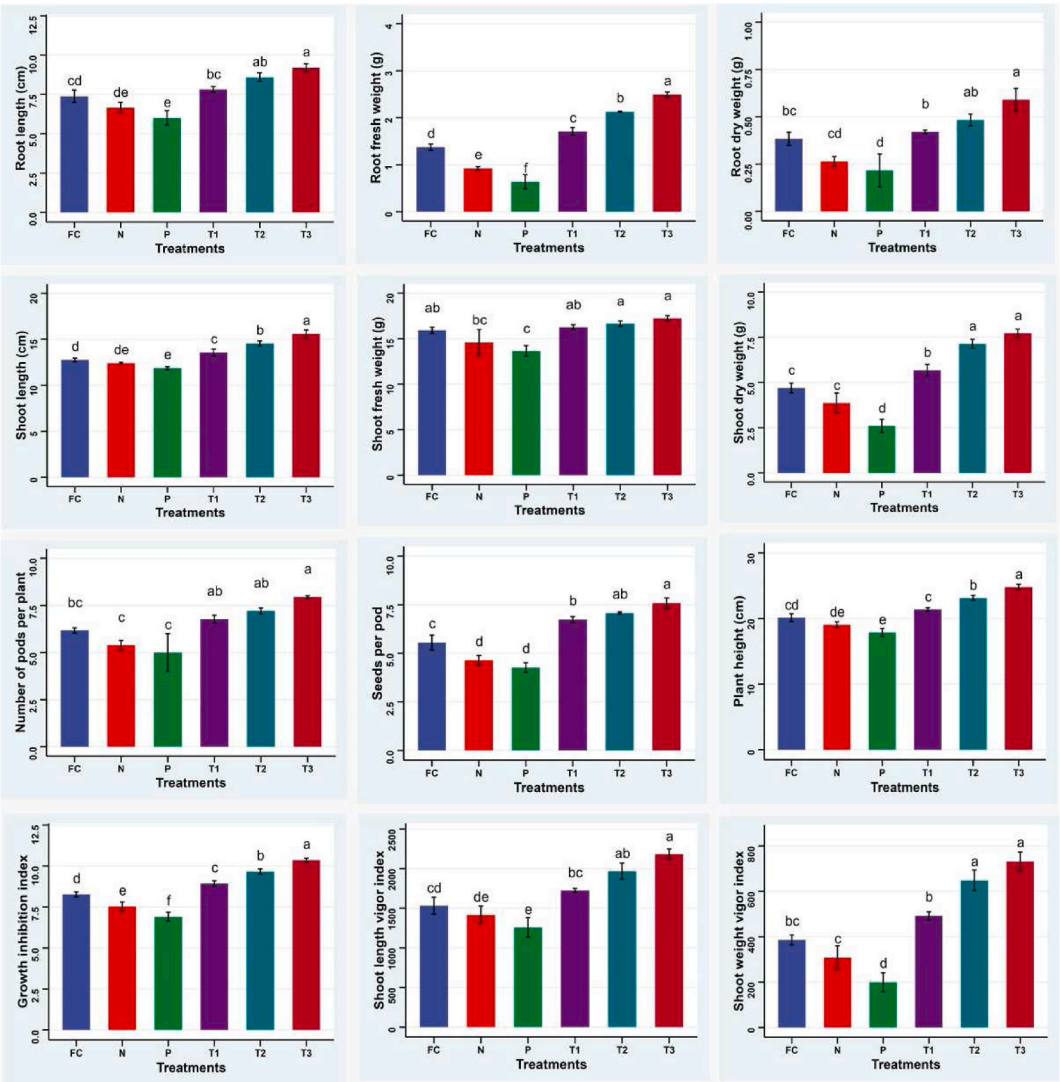

Fig. 4. Effect of *T. harzianum* metabolites con. on pea plant growth attributes with different treatments FC (Topsin M − 70 + Fop), N (-ive control (without pathogen and metabolites filtrate), P (+ ive control (*F. oxysporum* f. sp. *pisi*), T1 (40% metabolites filtrate + Fop), T2 (50% metabolites filtrate + Fop) and T3 (60% metabolites filtrate + Fop) (40%, 50%, and 60%) in susceptible germplasm (Meteor) against *F. oxysporum* f.sp. *pisi* 40 days after inoculation. Panels with different colors show different treatments from T1 to T6 where T6 represents control and means followed by the similar letters on each panel are not statistically different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(40–36%) in TPC for the healthy plants. Total flavonoid content (TFC) was significantly decreased in inoculated plants as compared to their respective healthy plants (40–36%). A significant increase in antioxidant enzymatic activities [(peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and catalase (CAT)] were observed in inoculated leaves as than healthy leaves at 40 and 80 days of inoculation. The increasing order of enzymes activities were as follow: CAT > POX > PPO > PAL in all treatments.

The maximum catalase activity was observed in the positive control as compared to T5 with 60% conc. Catalase activity increases (55–60%) in T5 after 40 and 80 days of inoculation. Peroxidase activity increased (52–59%) as compared to the positive control. An insignificant increase (66–77%) was observed in PPO activity in T5 as compared to all treatments. PAL activity in T5 exhibited a significant increase in PAL activity. Whereas this treatment showed a 59–64% increase in PAL activity (Figs. 7 and 8).

Biochemical and enzymatic analyses were utilized to generate a biplot graph after 40 and 80 days of inoculation. The principal analysis component (PCA) accounted for about 98.38% of the total variability, reflecting the excellent contribution of the two PC axes to the plotted components. Negative treatment and T1 are highly negatively correlated after 40 days, while negative control is strongly negatively correlated after 80 days. T3 and T1 showed a positive, strong correlation after 40 and 80 days, respectively. Total phenol contents with their long projection showed their higher variance, and they were at high Euclidean distance with the physiological attributes but still significantly and positively correlated. Moreover, PCA characterized nine different physiological parameters of the

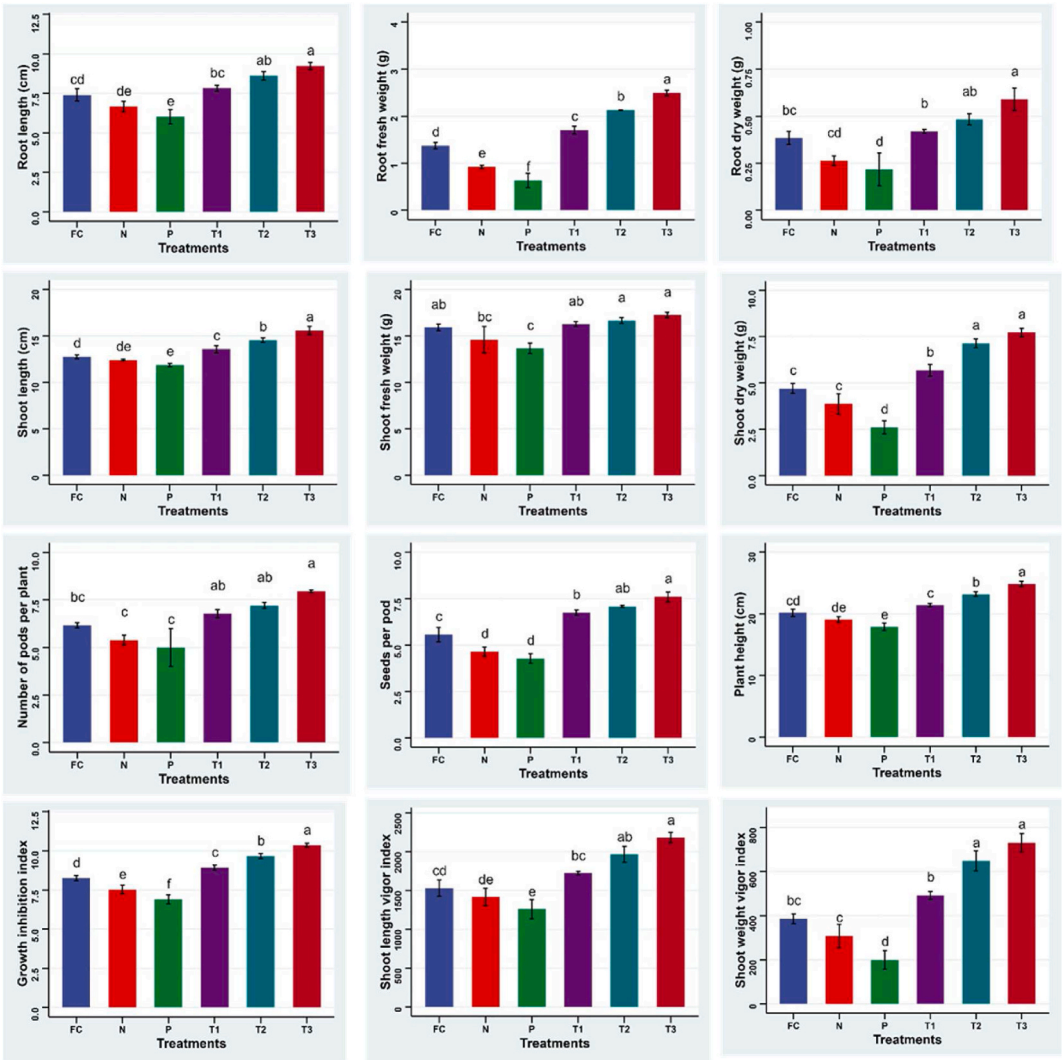

Fig. 5. Effect of *T. harzianum* metabolites concentration. on pea plant growth attributes with different treatments FC (Topsin M − 70 + Fop), N (-ive control (without pathogen and metabolites filtrate), P (+ ive control (*F. oxysporum* f. sp. *pisi*), T1 (40% metabolites filtrate + Fop), T2 (50% metabolites filtrate + Fop) and T3 (60% metabolites filtrate + Fop) (40%, 50%, and 60%) in susceptible germplasm (Meteor) against *F. oxysporum* f.sp. *pisi* 80 after inoculation. Panels with different colors show different treatments from T1 to T6 where T6 represents control and means followed by the similar letters on each panel are not statistically different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

biplot (Fig. 9A and B).

4. Discussion

F. oxysporum f. sp. *ciceris* is one of the most potent yield-limiting agents for peas (*Pisum sativum* L.) in the globe. It enters through the roots, and the germ tube penetrates plant epidermal cells. Eventually, the hyphae spread to the root cortical region and infiltrate the xylem vessels, preventing water and other essential solutes from being carried upward and causing wilt. Furthermore, the saprophytic fungus can remain in soil or detritus for up to six years, reducing yield [38]. As a result, substantial study has been conducted to find an efficient and practicable strategy to disease management, and a biological control-based approach has been identified as suitable [39]. Several efforts to treat soil or seed-borne illnesses have identified *Trichoderma* spp. as the most effective pathogen antagonist [40]. *Trichoderma's* antagonism against *Fusarium* spp. results from the coiling of antagonistic fungal hyphae, which causes lysis [41]. There is a need to highlight sustainable agricultural practices such as biological control management as an alternate or supplemental strategy that is environmentally beneficial, economically viable, and long-term [42]. Furthermore, antagonist microorganisms have the ability to secrete cell-wall degrading enzymes (chitinase and protease), which have biocontrol potential against many pre- and post-harvest fungal pathogens (*Alternaria* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizoctonia* sp., *Pythium* sp., *Bipolaris* sp.,

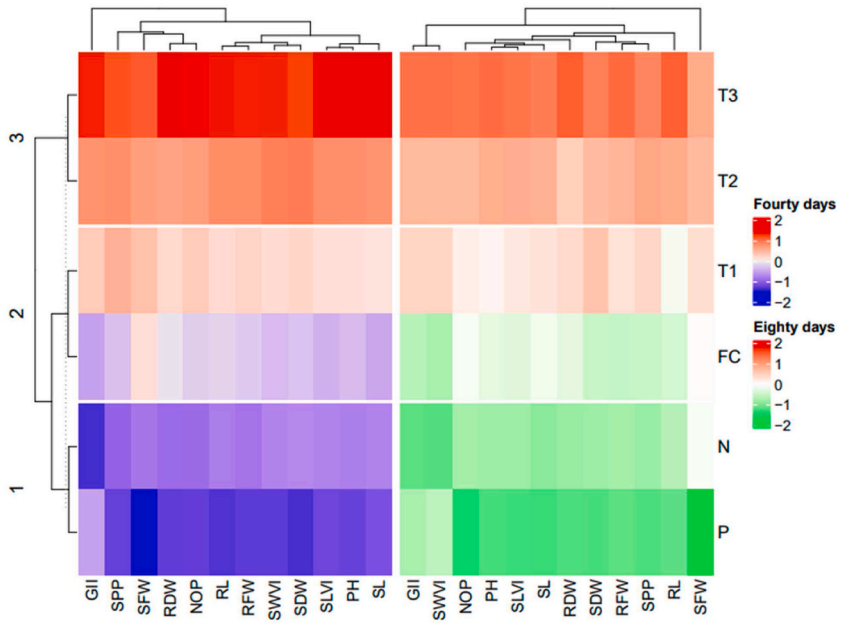

Fig. 6. Heatmap analysis of agronomic parameters of in-vivo experiment treatments after 40 and 80 days of inoculation.

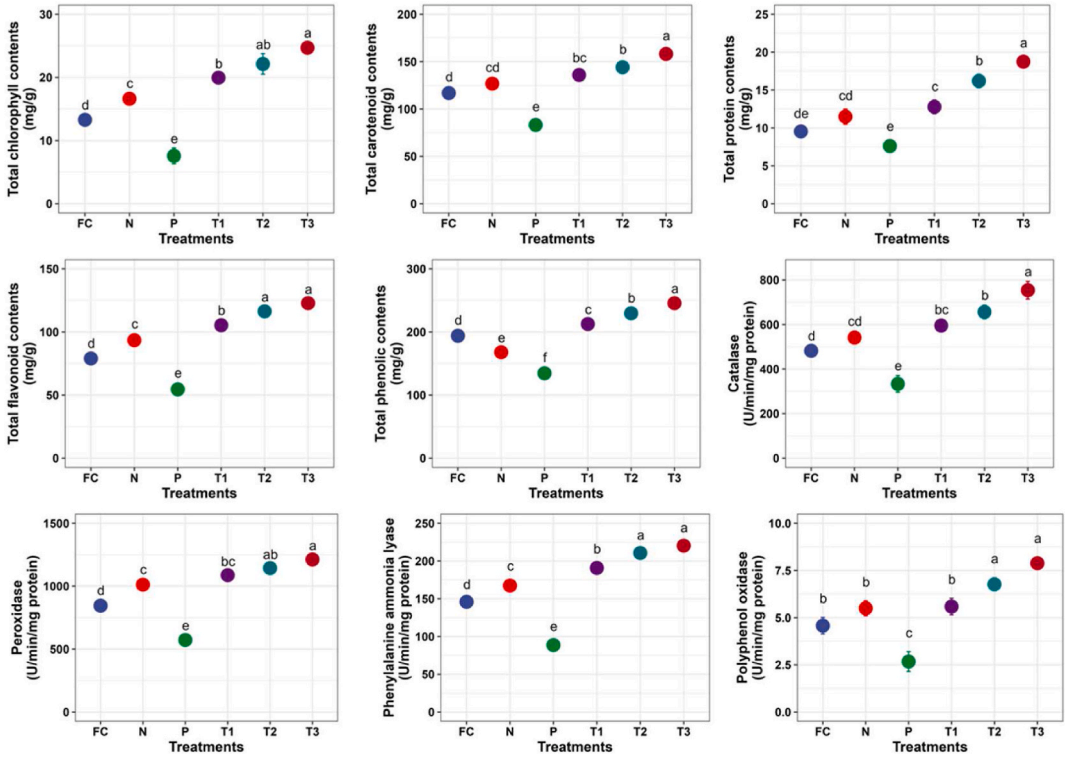

Fig. 7. Effect of *T. harzianum* metabolites con. on pea plant physiological attributes with different treatments FC (Topsin M − 70 + Fop), N (-ive control (without pathogen and metabolites filtrate), P (+ ive control (*F. oxysporum* f. sp. *pisi*), T1 (40% metabolites filtrate + Fop), T2 (50% metabolites filtrate + Fop) and T3 (60% metabolites filtrate + Fop) (40%, 50%, and 60%) in susceptible germplasm (Meteor) against *F. oxysporum* f.sp. *pisi* 40 days after inoculation. Panels with different colors show different treatments from T1 to T6 where T6 represents control and means followed by the similar letters on each panel are not statistically different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

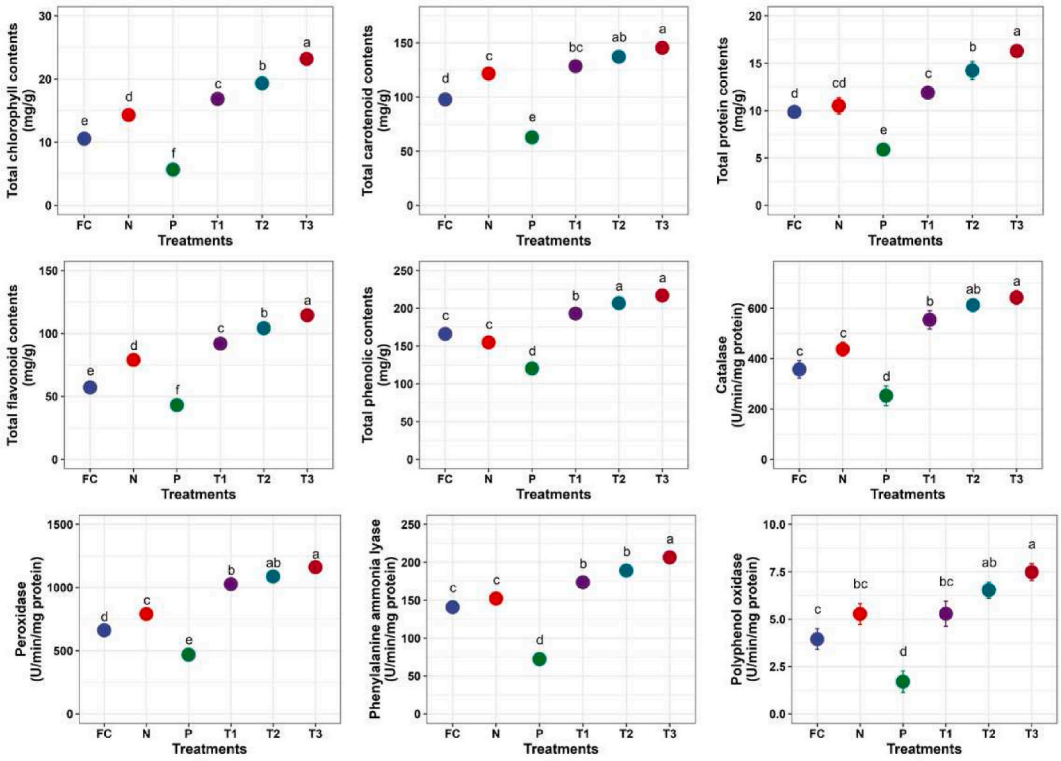

Fig. 8. Effect of *T. harzianum* metabolites con. on pea plant physiological attributes with different treatments FC (Topsin M − 70 + Fop), N (-ive control (without pathogen and metabolites filtrate), P (+ ive control (*F. oxysporum* f. sp. *pisi*)), T1 (40% metabolites filtrate + Fop), T2 (50% metabolites filtrate + Fop) and T3 (60% metabolites filtrate + Fop) (40%, 50%, and 60%) in susceptible germplasm (Meteor) against *F. oxysporum* f. sp. *pisi* 80 days after inoculation. Panels with different colors show different treatments from T1 to T6 where T6 represents control and means followed by the similar letters on each panel are not statistically different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

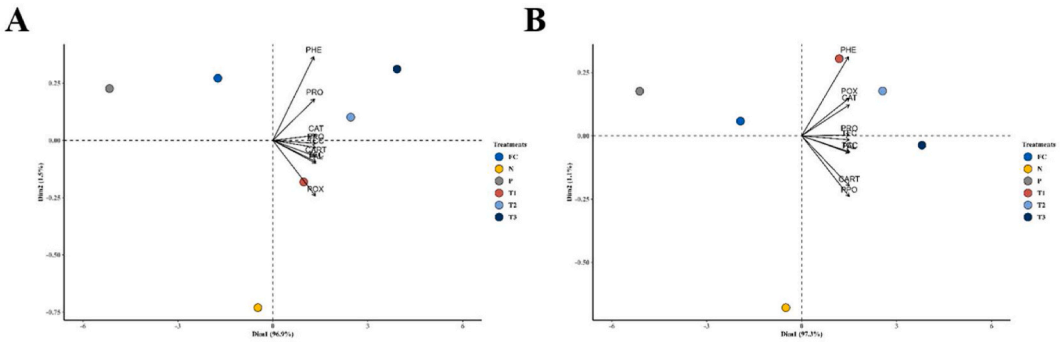

Fig. 9. A–B: Biplot of physiological traits of different treatments against *Fusarium oxysporum* for principal components analysis after 40 days (A) and 80 days (B) of inoculation.

Helminthosporium sp., *Macrophomina phaseolina*, *Sclerotinia sclerotium*, *Curvularia lunata*, *Botrytis cinerea*, and *Verticillium dahlia*) [43].

Current research aimed to evaluate the *Fusarium oxysporum* f. sp. pisi effect on pea crop growth and the physiochemical activities and efficiency of *Trichoderma* strains as a potential bio-control agent in managing Fusarium wilt. According to several studies, numerous species of *Trichoderma* have been discovered to produce various compounds that inhibit the growth and development of pathogenic fungi while also mycoparasitizing them in crops, such as *T. harzianumT. harzianum* exhibited (58–87%) inhibition in pathogen biomass, followed by *T. viridie* (56–78%), *T. longibrcatium* (64–83%), *Trichoderma koningii* (57–77%), and *T. hamatum* (52–73%). Variation in the antifungal potential of *Trichoderma* strain metabolites was due to differences in the production of pathogen cell wall degrading enzymes, chitinases and proteases [44]. The pathogen cell wall degrades due to the production of lytic enzymes. The authors of the reference [45] discussed that *T. viridie* and *T. harzianum* culture filtrates containing secreted proteins and

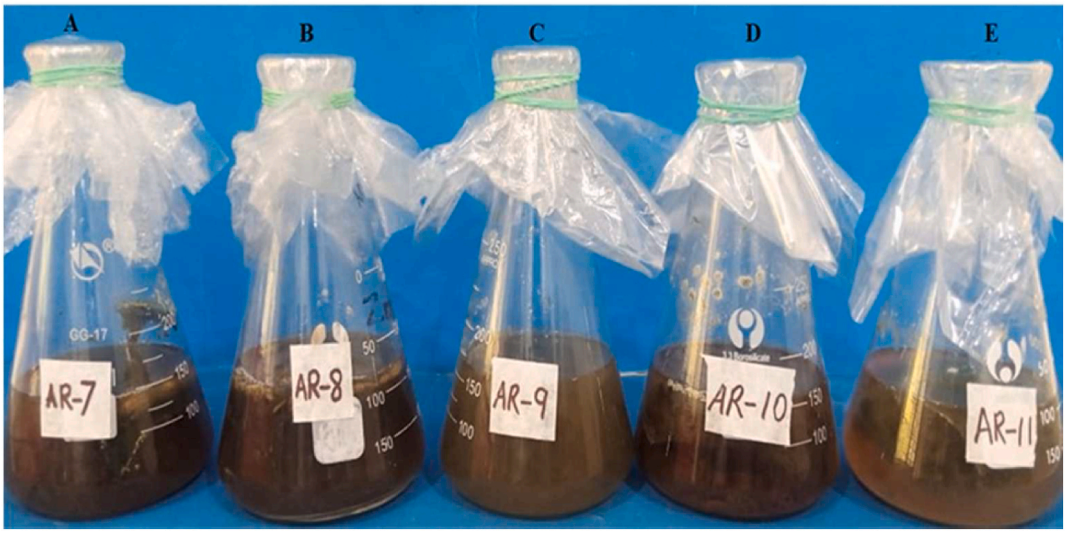

Fig. 10. A–E: Metabolites of five different Trichoderma spp after 30 days of incubation. (A) *Trichoderma koningi*, (B) *T. hamatum*, (C) *T. Longibrachitum*, (D) *T. viridie*, (E) *T. harzianum*.

Fig. 11. Comparison of *Fusarium* wilt disease on roots of different pea germplasms with respect to group of *fusarium* wilt disease rating scale. HR: Highly resistant, RR: Resistant, SS: Susceptible, HS: Highly susceptible.

Fig. 12. A–D: Effect of disease severity on pea plants (A) Sarsabz (B) Pea-09 (C) Supreem (D) Meteor.

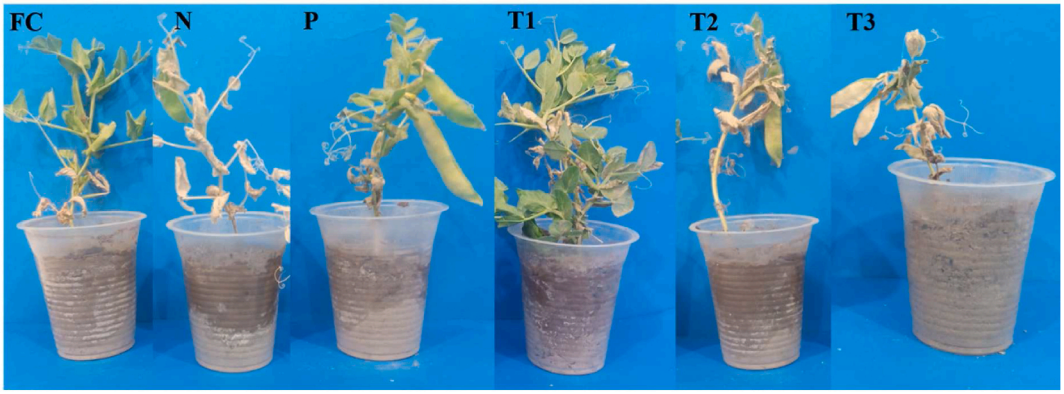

Fig. 13. Effect of *T. harzianum* metabolites concentration on pea plant with different treatments FC (Topsin M − 70 + Fop), N (−ive control (without pathogen and metabolites filtrate), P (+ive control (*F. oxysporum* f. sp. *pisi*)), T1 (40% metabolites filtrate + Fop), T2 (50% metabolites filtrate + Fop) and T3 (60% metabolites filtrate + Fop) (40%, 50%, and 60%) in susceptible germplasm (Meteor) against *F. oxysporum* f.sp. *pisi* 80 days after inoculation.

metabolites substantially inhibited (>75 percent decrease in growth) rhizosphere bacteria. Interaction of different concentrations of *T. harzianum* metabolites and the fungicide Topsin-M exhibited variable effects to mitigate disease, increase growth, and improve physiochemical attributes when under stress from *F. oxysporum* f. pisi inoculum. This study showed that treatment of the SS genotype with the positive control reduced growth and yield. The interaction with the highest concentration (60%) of *T. harzianum* metabolites and fungicides revealed maximum improvement in growth attributes. The authors of the reference [46] also reported that pea plants infected with *F. oxysporum* and cultivated in soil treated with compost and *T. harzianum* showed the greatest increase in growth and physiochemical parameters and a reduction in disease parameters. The authors of the reference [47] reported that *T. harzianum* produced peptaibols such as *Trichorzianines* A1 and B1 that act with cell wall-degrading enzymes to inhibit fungal pathogens. However, our results revealed that *T. harzianum* metabolites and antagonist interactions inhibit the pathogen and increase the growth of pea plants. The authors of the reference [48] found out that Trichoderma BCAs detected the presence of *F. oxysporum* by detecting particular *F. oxysporum* VCs as signals and increasing antifungal metabolite release [49–53].

5. Conclusion

Antifungal potential of *Trichoderma* species metabolites viz., *Trichoderma koningii*, *T. hamatum*, *T. longibratium*, *T. viridie*, and *T. harzianum* was evaluated against fusarium wilt of pea. Most effective Trichoderma strain was determined on in-vitro inhibition tests for the development of biocontrol formulations. Under field trials, all the metabolites had a certain level of potency against the pathogen, which increased as the metabolite concentrations increased. However, the older the metabolites, the higher the antimycotic ability was observed. Our findings concluded that *Trichoderma* spp. metabolites can be used as soil conditioning microorganisms and as potent biological control agents in integrated agricultural management.

5.1. Future prospects

The greatest issue in biocontrol management of soil-borne pathogenic fungi is ensuring a truly beneficial influence on the ecosystem. Although Trichoderma species are considered biocontrol agents in integrated plant disease management, their biocontrol capability remains limited to laboratory research. Furthermore, knowledge of its use is restricted and not widely circulated among farmers. Finally, genetic engineering and molecular techniques are required to develop biocontrol agents (BCAs) so that they can be utilized effectively against a diverse spectrum of soil-borne plant infections.

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Data availability statement

Data will be made available upon request

CRediT authorship contribution statement

Amna Rauf: Data curation, Conceptualization. **Muhammad Nasir Subhani:** Investigation, Data curation, Conceptualization.

Maroof Siddique: Writing – review & editing, Writing – original draft. **Habiba Shahid:** Writing – review & editing, Writing – original draft. **Muhammad Bilal Chattha:** Writing – review & editing, Writing – original draft. **Abdulwahed Fahad Alrefaei:** Writing – review & editing, Methodology. **Syed Atif Hasan Naqvi:** Writing – review & editing, Investigation. **Haider Ali:** Writing – review & editing, Visualization, Validation, Funding acquisition. **Rosa Sanchez Lucas:** Writing – review & editing, Visualization, Validation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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